CHAPTER 8

Chemical Constituents of the Fungal Cell

1. Elemental Constituents and Their Roles

VIRGIL GREENE LILLY

Department of Plant Pathology, Bacteriology, and Entomology, West Virginia University Morgantown, West Virginia

I. INTRODUCTION

Chemical analyses to have meaning should be related to the species, age, and type of cells analyzed, the composition of the medium in which the cells were grown, and environmental factors. Fungus cells do not have an unvarying composition.

Relatively few complete analyses of the mineral elements (ash) found in fungus cells have been reported. Richards and Troutman (1940) made a spectroscopic examination of the mineral elements present in the ash of yeast cells grown in various media. They found: Ba, Bi, B, Ca, Cr, Cu, Au, Fe, La, Pb, Mg, Mn, P, Pt, K, Ag, Na, Tl, Sn, and Zn. Recently, Grant and Pramer (1962) analyzed quantitatively five samples of yeast extract and found Al, Ba, Cd, Co, Cr, Cu, Fe, Ga, Mg, Mn, Mo, Ni, Pb, Sn, Sr, Ti, V, and Zn. Some of these elements may not have been present in the original yeast cells.

The composition of fungus cells may be made to vary widely. Conway and Moore (1954) subjected yeast cells to repeated fermentations in a medium containing glucose and sodium citrate; 98% of the potassium was replaced by sodium after seven 2-hour fermentations. By fermenting a "sodium yeast" in a glucose-magnesium acetate medium most of the sodium and potassium was replaced by magnesium. The magnesium yeast contained 3, 2.6, and 292 meq/kg wet yeast of potassium, sodium, and magnesium, respectively (Conway and Beary, 1962).

II. ESSENTIAL ELEMENTS

At least a third, perhaps half, of the elements have been found in fungus cells. Analysis in itself does not reveal which elements are essential, or the functions of essential elements in the living cell. An essential element is one that is indispensable. Nicholas (1957, 1961) would replace the term "essential element" by "metabolic nutrient." More importantly, he distinguishes between the classical and the functional methods of determining essentiality.

A. The Classical Method

The classical method uses a highly purified medium, complete in all respects save one. When the missing essential element is added to such a medium, the test fungus grows. In addition, the response of the test fungus should be proportional, within limits, to the concentration of the essential element in question.

There is no difficulty in showing that those elements required in high concentrations are essential. These include carbon, nitrogen, phosphorus, potassium, and magnesium. Difficulties arise when low concentrations of an element are required. These elements are called variously micro, trace, or minor elements and include iron, zinc, manganese, copper, calcium, and possibly others.

The primary problem in the classical method is to remove completely, or to reduce to very low levels, the metallic contamination in the medium. Only then can the increased growth be attributed to the element under study.

B. Various Methods of Removing Metallic Contamination

Steinberg (1935) autoclaved the complete medium with calcium carbonate, which was then filtered off. Nicholas (1952) used a variety of methods including precipitation with hydrogen sulfide (Cu and Mo), complex formation with 8-hydroxyquinoline followed by extraction with chloroform (Zn, Fe, and Ga), and coprecipitation of copper and manganese. Media so purified still contained traces of metals: copper, 1 μg/l; iron, zinc, manganese, and gallium, 0.2 μg/l; and molybdenum, 0.1 m μg/l. See Donald *et al.* (1952) for a comparison of 15 methods of removing metallic contamination from media.

The early investigators were hampered by the solubility of the chemical glassware then available (Javillier, 1914). Quartz vessels are considered

to be the most desirable, but Pyrex glass is satisfactory. Plastic culture vessels have been used; presumably polypropylene vessels which can be autoclaved would be satisfactory.

There is an extensive literature on the metal ion requirements of fungi, including reviews by Steinberg (1939b), Foster (1939, 1949), Perlman (1949), Lavollay (1955), Lilly and Barnett (1951), Hawker (1950), and Cochrane (1958).

C. The Functional Method

The basic approach of the functional method is to show that an element plays an indispensable role in some enzyme system isolated from a fungus (or other organism). The classical method is usually employed first; the more sophisticated method can then be used to answer questions that the classical method cannot. If it can be shown that a fungus contains an essential enzyme system that requires a specific metal as a constituent or activator, this is excellent proof that the metal is essential (see pages 171–173).

The use of electron paramagnetic resonance spectroscopy to study the function of the transition metals in biological systems is relatively new. Nicholas *et al.* (1962) used this method to study iron, manganese, molybdenum, and copper complexes in bacteria. Presumably, the method is applicable to fungi as well.

III. BIOLOGICAL SUBSTITUTION

If one element completely replaces another the concept of an essential element is in need of modification and amplification. The total replacement of one element by another has rarely been reported in fungi. Ingraham and Emerson (1954) reported that strontium completely replaced calcium in the nutrition of *Allomyces arbuscula,* strain Burma lDb. However, some ten times as much strontium was required as calcium. Partial biological substitution of sodium for potassium in *Aspergillus niger* was reported by Steinberg (1946). No evidence was presented to indicate that sodium could completely replace potassium. In nature, or on complex natural media, partial biological substitution may be more common than usually thought (see the interesting discussion by MacLeod and Snell, 1951).

IV. ION ANTAGONISM

The concentration of an essential ion required may depend upon the concentration of other ions in the medium. While this phenomenon is of

great interest in cultural studies, it may be assumed that the composition of the cells is also affected. MacLeod and Snell (1951) found competition between potassium and sodium ions in the growth of *Saccharomyces carlbergensis*. As the concentration of sodium ions was increased it was necessary to increase the concentration of potassium ions to obtain an equivalent amount of growth. Abelson and Aldous (1950) found the amounts of cobalt and nickel accumulated by cells of *Torula* [*candida*] *utilis* to be dependent on the magnesium concentration of the medium.

V. ESSENTIAL NONMETALLIC ELEMENTS

The nonmetals hydrogen, carbon, nitrogen, oxygen, phosphorus, and sulfur are essential for all fungi. Some fungi metabolize, or accumulate, arsenic, selenium, chlorine, silicon, and other nonmetals.

A. Hydrogen

Hydrogen is the most abundant element in the universe, both on a weight and on an atom per cent basis. On the atom per cent basis, hydrogen is the most abundant element in the cells of fungi and other organisms. However, most analytical data are reported on the basis of weight per cent, a method that obscures the relative numbers of different atoms found in cells.

Hydrogen is a structural and functional element. This element is contained in all the organic compounds found in cells. For some of the transformations of organic compounds in the living cell see Chapters 10 to 12.

The hydrogen content of *dry* cells is seldom reported. From the standpoint of proximate analysis, fungus cells are mainly carbohydrate, protein, fat, and ash. Presumably, an average value for fungus cells would lie between 6 and 8%. Hilpert *et al.* (1937) reported *Aspergillus niger* mycelium to contain 6.7% hydrogen.

1. Water

Fungus cells (excepting some spores) are mainly water. Hawker (1950) cites data on the water content of various sporophores which ranged from 73.7% (*Pleurotus ostreatus*) to 93.85% (*Amanita rubescens*). The water content of mycelium grown in liquid medium is difficult to measure exactly because it is difficult to judge when the surface water has been removed.

Spores, in general, contain less water than mycelium. Yarwood (1950) determined the water content of the asexual spores of 9 species of fungi:

Penicillium digitatum, 6.0%; *Uromyces appendiculatus*, 12%; *Aspergillus niger*, 13%; *Peronospora destructor* and *Botrytis cinerea*, 17%; *Monilinia* (*Sclerotina*) *fructicola*, 25%; *Erysiphe cichoracearum*, 52%; *E. polygoni*, 72%; and *E. graminis*, 75%. The high water content of spores of *Erysiphe* spp. is not typical of most others.

2. Bound Water

Water exists in fungus cells in two forms. Free water is that removed from cells by drying at room temperature over a desiccant in a vacuum. Bound water is that removed by additional drying at elevated temperatures (80°–110°C). Todd and Levitt (1951) measured the bound water content of mycelium of *Aspergillus niger* cultivated in media containing from 50 to 410 g of glucose per liter. The bound water content increased about tenfold as the glucose concentration increased. Presumably, bound water is essential in the life processes of fungi, and the concentration varies with the environment.

B. Carbon

Raistrick *et al.* (1931) made extensive studies of *Aspergillus*, *Penicillium*, and *Fusarium*. The extreme carbon values for *Aspergillus* were 56.6% (*A. sydowii*) and 43.5% (*A. parasiticus*); for *Penicillium*, 62.9% (*Scopulariopsis brevicaulis*) and 45.3% (*Penicillium viridicatum*); and for *Fusarium*, 61.2% (*F. rhizophilum*) and 49.0% (*F. lini*).

Whitaker (1951) determined the carbon content of the mycelium of 40 species, representing 21 genera of wood-rotting fungi. In general, the mycelium of this group of fungi contained less carbon than those studied by Raistrick *et al.* (1931). The effects of continuous and restricted aeration on the carbon content of the mycelium were compared for a number of species. The carbon content of *Peniophora gigantea* under restricted aeration was 44.1%, and under continuous aeration 40.0%. On the other hand, the mycelium of *Fomes fomentarius* contained 40.7% carbon under restricted aeration, and 44.3% under continuous aeration.

C. Nitrogen

The nitrogen content of fungus cells is variable and depends on species, age, and composition of the medium. Nielsen and Schneider (1957) grew *Rhodotorula gracilis* in media containing 1 and 10 g of asparagine per liter. The nitrogen content of the cells was 1.34 and 9.72% in the low and high asparagine media, respectively. Similar results were obtained with media

containing 1 and 10 g of ammonium sulfate per liter. The nitrogen content was 2.94 and 7.00% in the low and high ammonium sulfate media, respectively [see Cochrane (1958) for additional citations].

D. Oxygen

No direct analyses of mycelium for oxygen appear to have been published. An estimate, based on carbohydrate, protein, and fat content, would lie in the range of 25–35%. Most of the organic compounds found in cells contain oxygen. See Chapter 12 for information on the role of oxygen in respiration.

E. Phosphorus

This element is usually the most abundant nonmetallic element found in fungus ash. Analytical results in the literature are often expressed as percentages of phosphorus pentoxide (P_2O_5 contains 43.64% P). The literature has been reviewed by Foster (1949) and Cochrane (1958).

Rennerfelt (1934) studied the salt uptake of *Aspergillus niger* under a variety of nutritional conditions. The phosphorus content of mycelium grown in medium containing 1/2500 mole KH_2PO_4 per 100 ml was 0.014 mmole/gm; when the phosphate content of the medium was increased tenfold, the mycelium contained 0.095 mmole of phosphorus per gram. Increasing the phosphate content of the medium to still higher levels increased the phosphorus content of the mycelium slightly. Bajaj *et al.* (1954) found inorganic and organic phosphates to be easily leached from the spores of *A. niger*. The phosphorus content of spores was 3–4 times that of the mycelium on which they were borne (Rennerfelt reported that spores of *A. niger* contained 1.4 times as much phosphorus as mycelium). Young mycelium was found to contain more phosphorus than old; in one experiment the phosphate content of 3-, 6-, and 9-day-old mycelium was 12.7, 4.9, and 2.4 μg/mg, respectively.

The roles of phosphorus in metabolism and energy transfer are discussed in Chapter 10.

F. Sulfur

The sulfur content of fungus cells is variable; it is dependent on the concentration of this element in the medium, or substrate, and on other factors. Nielsen and Lundin (1955) found the nitrogen content of the medium to influence the sulfur content of the cells of *Rhodotorula gracilis*. Presumably, this was due in part to the increased protein content of the

cells, since most proteins contain methionine and cysteine. Cells from the high nitrogen medium contained 0.53% sulfur; those from the low nitrogen medium contained 0.11%. These investigators also found that the sulfur content of the medium influenced that of the cells. When the medium contained 0.5 g of sulfur per liter, the cells contained 0.21% sulfur; and when the sulfur content of the medium was reduced to 0.0025 g/l, the sulfur content of the cells was 0.10%.

The sulfur-containing compounds found in mycelium include enzymes and other proteins, the amino acids cysteine and methionine, the tripeptide glutathionine, and two vitamins, thiamine and biotin.

VI. OTHER NONMETALLIC ELEMENTS

Fungi metabolize, or accumulate, nonessential elements. Holzapfel and Engel (1954) cultured *Aspergillus niger* on a special medium containing high concentrations of silicon (280–330 mg SiO per liter). The surface mycelium contained about 2%, and the submerged mycelium contained about 9%, silica. Selenium appears to be metabolized by *A. niger* (Weissman and Trelease, 1955); the organic selenium compounds formed were not identified. *Scopulariopsis brevicaulis* synthesizes arsine from arsenic trioxide (Challenger *et al.*, 1954). A number of fungi synthesize organic chlorine compounds, e.g., griseofulvin. For a review, see Foster (1949).

VII. ESSENTIAL METALLIC ELEMENTS

The following metallic elements appear to be essential for all fungi tested: potassium, magnesium, iron, zinc, manganese, copper, and molybdenum. Calcium is essential for some, but not all, fungi. Restricted evidence is available for the essentiality of a few other metals.

Many of the essential metals may be assumed to function as enzyme activators or as constituents of enzymes. The effects of suboptimal concentrations are manifested in various ways: reduced growth, decreased sporulation, increased or decreased concentrations of various enzymes in the cells, and increased or decreased synthesis of various metabolites. Many of the essential elements are toxic in too high a concentration.

A. Potassium

Spores and mycelium usually contain more potassium than any other metal. Rennerfelt (1934) found spores of *Aspergillus niger* to contain 4.9 times as much potassium as did mycelium. Rippel and Behr (1934) analyzed the mycelium of 5 isolates of *A. niger* and found the potassium con-

tent to depend on age of the culture, potassium content of the medium, and the isolate. Young mycelium contained more potassium than old mycelium. The highest potassium content found was 2.95%. Rosselet (1953) used *A. niger* to assay the available potassium content of soil.

Nakamigawa and Okuda (1960) found *Mucor mandschuricus* to accumulate pyruvic acid in a medium lacking thiamine, but containing potassium or rubidium ions. Pyruvic acid accumulated when the medium contained thiamine and rubidium, but not when both thiamine and potassium were present. If pantothenic acid and thiamine were added to a medium containing rubidium, pyruvic acid did not accumulate.

B. Magnesium

This element appears to be essential for all fungi. Nicholas and Fielding (1951) found the growth of *Aspergillus niger* to be proportional to the magnesium content of the medium; 20 mg/l was required for maximum production of mycelium. Vail and Lilly (1961) found *Phycomyces blakesleeanus* to tolerate magnesium concentrations of 820 mg/l without inhibition of growth, although carotene production was depressed at this concentration. *Allomyces arbuscula* required magnesium for growth; 9 mg/l sufficed for maximum growth and 200 mg/l was toxic (Ingraham and Emerson, 1954).

Rippel and Behr (1930) found the magnesium content of *Aspergillus niger* mycelium to be dependent on the age of the culture and on whether the medium contained ammonium or nitrate nitrogen. Three-day-old mycelium grown in the ammonium-nitrogen medium contained 0.13% magnesium, whereas comparable mycelium grown in the nitrate-nitrogen medium contained 0.265% magnesium. Rennerfelt (1934) found the spores of *A. niger* to contain 7.1 times as much magnesium as did mycelium.

Conway and Beary (1962) prepared a yeast containing 292 meq of magnesium per kilogram wet cells and found this "magnesium yeast" to grow slowly in the absence of potassium. The cells of the "magnesium yeast" were abnormal, being elongated and associated in chains.

VIII. MICROELEMENTS

Iron, zinc, manganese, copper, and molybdenum are variously called micro, minor, and trace elements because they are required in low concentrations. Calcium and perhaps other elements may be included in this group. Numerous reports in the literature state that this or that micro-

element is not required by a particular fungus. Judgment is required in evaluating negative results.

A. Iron

This element is essential for all fungi. As little as 0.1 μg of iron in 50 ml of medium may be detected by *Aspergillus niger* (Nicholas, 1952). The classical method of determining a requirement for iron is supported by the functional approach, since this element is a constituent of various enzymes including the cytochromes, cytochrome oxidase, catalase, and others.

Ferrous or ferric ions satisfy the iron requirements of most fungi. *Piloboolus* requires iron to be supplied as various chelated iron compounds (heme, coprogen, or ferrichrome) according to Page (1962). For a review of chelated iron compounds in nutrition, see Neilands (1957).

B. Zinc

This element, like iron, appears to be essential for all fungi. Zinc is a constituent, or activator, of a number of enzymes. Alcohol dehydrogenase contains 4 atoms of zinc per molecule (Vallee and Hoch, 1955). Zinc is essential for the functioning of this enzyme.

Grimm and Allen (1954) found a zinc concentration of 0.001 ppm to be sufficient for optimal growth of *Ustilago sphaerogena*. The cells were colorless when grown in media containing this concentration of zinc, but were pink when cultured in a sucrose-yeast extract medium. The pink cells contained as much as 1% cytochrome c. High yields of cytochrome c were obtained when the sporidia were cultured in a synthetic medium containing 1 ppm of zinc, 2 ppm of thiamine, and ammonium nitrogen. Thus, the concentration of zinc influenced the synthesis of cytochrome c, an iron-containing enzyme.

The reduced growth and sporulation of fungi cultured in media containing suboptimal concentrations of zinc (and other essential microelements) is readily apparent. Other effects may be detected by different techniques. Nason *et al.* (1951) cultured *Neurospora crassa* in a medium containing sufficient zinc for half-maximum growth, and also in the basal medium fortified with additional zinc. As expected, alcohol dehydrogenase was found in mycelium cultured in the medium containing added zinc; this zinc-containing enzyme was not detected in 4 out of 5 samples of mycelium grown in the low-zinc medium. The concentration of tryptophan synthetase was much lower in mycelium grown in the medium to which

zinc was not added than when adequate concentrations of this element were present. The DPNase content of the zinc-deficient mycelium was 10–20 times greater than in the control mycelium. Moderate zinc deficiency did not affect the concentration of fumarase.

C. Manganese

A requirement for manganese is difficult to establish for some fungi using classical methods. Nicholas (1952) found the mycelial weight of *Aspergillus niger* to be little affected by the omission of manganese from the medium, but very few spores formed in the absence of added manganese. Donald *et al.* (1952) reported a 50% reduction in growth of *A. niger* when manganese was omitted from media purified by certain techniques. These authors also showed that different isolates of *A. niger* varied in their requirements for manganese.

By using the functional method, Medina and Nicholas (1957) demonstrated that hydroxylamine reductase from *Neurospora crassa* requires manganese for activity. In addition, mycelium grown in media low in manganese contained 57% as much hydroxylamine reductase as "normal" mycelium. Mycelium of *N. crassa* grown in media low in manganese also contained reduced concentrations of nitrite and hyponitrite reductases.

D. Copper

The essential nature of copper has been demonstrated by classical methods. Steinberg (1950) found that the omission of copper from a highly purified medium resulted in a reduction of growth of *Sclerotium rolfsii, Cercospora nicotianae, Fusarium oxysporum, Pythium irregulare,* and *Thielaviopsis basicola.* Nicholas (1952) found the growth of *Aspergillus niger* to be proportional, within limits, to the copper concentration of the medium. As little as 0.05 μg/50 ml could be detected. Many investigators have noted that the spore color of *A. niger* changes from yellow to brown and then to black as the copper concentration is increased.

A number of enzymes, including tyrosinase and laccase, contain copper. Medina and Nicholas (1957) showed that copper is involved in the biosynthesis of nitrite and hyponitirite reductases.

E. Molybdenum

Steinberg (1936, 1937), using the classical method, found that *Aspergillus niger* required more molybdenum when cultured on a medium containing nitrate nitrogen than when ammonium nitrogen was used. Ex-

tremely low concentrations of molybdenum are detected by *A. niger*. Nicholas and Fielding (1950) found the addition of 0.1 mμg of molybdenum to 50 ml of purified medium resulted in an increase of 57 mg of mycelium. For other fungi that require molybdenum, see Steinberg (1950).

Molybdenum is known to be required for the formation and activation of nitrate reductase in fungi (and other organisms). Nicholas *et al.* (1953) cultured *Neurospora crassa* and *A. niger* in the absence and presence of added molybdenum. In all experiments, the omission of added molybdenum reduced the amount of nitrate reductase synthesized. Nicholas and Nason (1954) showed that *N. crassa* nitrate reductase is a flavoprotein containing molybdenum. Nicholas (1957) found the nitrate reductase content of mycelium of *N. crassa* grown in the absence of added molybdenum to be 10% of that found in mycelium grown in its presence. Growth in the absence of added molybdenum was 29% of the control. Nitrate reductase is an induced enzyme, little or none being synthesized by *A. niger* and *N. crassa* grown in media containing ammonium nitrogen. Molybdenum was not replaced by iron, zinc, manganese, nickel, silver, tungsten, chromium, vanadium, cobalt, or boron.

Medina and Nicholas (1957) summarized the steps in the reduction of nitrate to ammonia by *N. crassa* as follows:

$$\text{Nitrate} \xrightarrow{\text{Mo}} \text{nitrite} \xrightarrow{\text{Cu,Fe}} \text{hyponitrite} \xrightarrow{\text{Cu,Fe}} \text{hydroxylamine} \xrightarrow{\text{Mn}} \text{ammonia}$$

F. Calcium

Steinberg (1948) reinvestigated the calcium requirements of fungi and concluded that this element was not required by *Aspergillus niger* or *Fusarium oxysporum* var. *nicotianae*. *Alternaria solani* and other species made reduced growth in the absence of added calcium. For a review of the early literature, see Rippel and Stoess (1932).

Calcium does not appear to be required for the growth of *Chaetomium* sp., but it frequently increases the formation of perithecia (Basu, 1951). More investigation will be required to determine the role of calcium in fungi.

G. Scandium, Vanadium, and Gallium

Steinberg (1939a) found *Aspergillus niger* to make increased growth in a glycerol medium when scandium was added. Steinberg (1950) reported that scandium partially replaced calcium for *Alternaria solani*. Vanadium appeared to be essential for an isolate of *A. niger* (Bertrand, 1941). Omission of gallium from a highly purified medium led to a reduc-

tion in growth and sporulation by an isolate of *A. niger* (Steinberg, 1938). Under some conditions, growth of *Corticium solani* and *Pythium irregulare* was reduced when gallium was omitted from the medium (Steinberg, 1950).

H. Essential Elements Not Required by Fungi

Boron, fluorine, sodium, chlorine, iodine, sodium, cobalt, and vanadium are essential for various organisms, but none of them appear to be required by fungi.

I. Nonessential Metals

Often fungi are in the presence of, and may absorb, nonessential metals. Presumably, none of these metals are inert, and in sufficient concentration they may be toxic. The heavy metals as fungicides are treated in Chapter 20. A few examples of the effects of nonessential metals on particular fungi are noted below.

Lilly *et al.* (1962) found sodium and lithium chlorides to inhibit germination of unheated sporangiospores of *Phycomyces blakesleeanus*. Lithium chloride (4.3 mmoles/l) inhibited growth of *P. blakesleeanus,* whereas the same concentration of sodium chloride did not. Cobalt is not essential for any fungus, so far as is known, but Nicholas (1952) reported that *Aspergillus niger* synthesized vitamin B_{12} when cobalt was added to the medium. Mandels and Reese (1957) noted that the production of cellulase by *Trichoderma viride* increased when calcium (30 mg/l) was added to an unpurified medium when cobalt was present.

IX. SUMMARY

The elements essential for all fungi include hydrogen, carbon, nitrogen, oxygen, phosphorus, sulfur, potassium, magnesium, iron, zinc, manganese, copper, and molybdenum. Some fungi require calcium. The actual composition of fungi growing in nature, or on nonpurified media, may contain 2 to 3 times as many elements as are listed above. The following elements comprise the greater part of fungus cells: hydrogen, oxygen, carbon, nitrogen, phosphorus, sulfur, potassium, and magnesium. Iron, zinc, manganese, copper, and molybdenum occur in low concentrations in fungus cells and exert their physiological effects as components or activators of enzymes. The nonessential elements may be accumulated; in sufficient concentration many of them are toxic.

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